Effect of Heparin Administration on Metabolomic Profiles in Samples Obtained During Cardiac Catheterization

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Background—Metabolic profiling holds promise for early detection of coronary artery disease and assessing risk for ischemic events. Heparin is frequently administered (1) to treat acute coronary syndromes; and (2) during routine cardiac catheterization procedures. Because it stimulates lipolysis, heparin is a potential confounder of metabolic profiling in these populations.

Methods and Results—Using mass spectrometry and conventional immunoassays, we evaluated how unfractionated heparin administration affected 69 peripheral blood metabolites (acylcarnitines, amino acids, nonesterified fatty acids and their oxidation byproducts, conventional lipids, glucose, and C-reactive protein) in samples obtained pre- and postcardiac catheterization from 19 patients who received heparin and 10 patients who did not. Using unpaired t tests, we compared the changes in mean metabolite levels before and after the procedure between the nonheparin and heparin groups. Clinical characteristics of the nonheparin and heparin groups, indication for cardiac catheterization, procedure performed, and other periprocedural variables were similar. The mean change between pre- and postprocedure β-hydroxybutyrate (5.43 versus 66.84 μmol/L; P=0.009), ketones (21.17 versus 98.49 μmol/L; P=0.009), nonesterified fatty acids (0.37 versus 1.20 mmol/L; P=0.017), and triglycerides (~9.33 versus ~36.50 mg/dL; P=0.007) was significantly different between the nonheparin and heparin groups, respectively. There were no significant differences between groups in the other metabolites measured.

Conclusions—Heparin administration during cardiac catheterization induced changes in peripheral blood metabolites that were consistent with known lipolytic effects of heparin and define a metabolite signature associated with heparin administration. These findings are important for accurate interpretation of future metabolic profiling studies in populations exposed to heparin. (Circ Cardiovasc Genet. 2011;4:695-700.)

Key Words: cardiac catheterization ■ coronary artery disease ■ heparin ■ metabolic profiling

Metabolomics is an emerging field that comprises high-throughput identification and quantification of small molecule metabolites.1,2 Profiling low-molecular-weight biochemicals, including lipids, sugars, and amino acids that serve as substrates and products in metabolic pathways, is particularly relevant to human conditions such as coronary artery disease (CAD). For example, open platform mass spectrometry analysis and functional pathway trend analysis detected changes in selected metabolites that correlated with ischemia during exercise stress testing.3 In addition, metabolomic profiling has identified changes in circulating levels of metabolites in patients undergoing planned myocardial infarction with alcohol septal ablation.4 Our group demonstrated that peripheral blood metabolites are heritable in families burdened with early-onset CAD.5 Furthermore, we showed that metabolic profiles discriminate subjects with CAD from CAD-free control subjects and identify patients at risk for future cardiac events.6 Thus, metabolomic profiling holds promise for early detection of CAD, early identification of myocardial infarction, and risk stratification.

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There are concerns that human metabolomic studies may be confounded by clinical and/or environmental influences such as diet and drug effects. Indeed, an early study reported that proton nuclear magnetic resonance analysis of plasma could discriminate angiographically defined advanced CAD with >90% accuracy and specificity.7 However, these findings were later refuted and were likely confounded by concomitant statin therapy.8

Similarly, heparin administration may be an important confounder of metabolomic analyses in patients with CAD in...
whom heparin is used frequently in both stable (eg, routine use in the catheterization laboratory) and acute (eg, acute coronary syndrome and suspected acute coronary syndrome) settings. Heparin administration causes the release of endothelial and hepatic lipoprotein lipase (LPL), thereby promoting hydrolysis of triglycerides in chylomicrons and very-low-density lipoprotein into 2 nonesterified fatty acids (NEFAs) and monoacylglycerol.9 Standard doses of heparin confound metabolomic analysis of selected oxylipids.10 It is unknown to what extent these effects modulate the metabolomic profiles obtained from plasma or serum of patients who receive heparin. The aim of our study was to characterize the effect of heparin on selected peripheral blood metabolite levels to assist in interpreting the results of cardiovascular disease metabolic profiling studies.

Methods

Study Population
In June 2009, 29 patients, aged >18 years, presenting to Duke University Medical Center for cardiac catheterization who had consented to participate in the CATHGEN biorepository were enrolled in the present study. The CATHGEN biorepository includes patients recruited sequentially through the cardiac catheterization laboratories at Duke University Medical Center. As part of the CATHGEN biorepository, demographic and clinical data, cardiac risk factors, procedure results, and longitudinal follow-up are archived through the Duke Databank for Cardiovascular Disease. In addition, 60 mL of blood for plasma, DNA, and RNA is collected at the time of arterial or venous sheath insertion. All patients fasted for ≥6 hours before the procedure.

The Duke University Institutional Review Board approved the protocol for the CATHGEN biorepository. For our study, patients also provided written informed consent for collection of an additional 3 mL of blood in EDTA-treated tubes at the conclusion of their catheterization procedure and before the access site sheath was removed or before percutaneous coronary intervention, if performed. The Duke Institutional Review Board approved the protocol for the additional blood collection and the use of baseline blood samples and clinical data for this study.

In our cardiac catheterization laboratory, it is routine clinical practice for most operators to administer 1 dose of intravenous unfractionated heparin after arterial sheath insertion. Our study did not influence whether heparin was given or how much heparin was given. There was no prospective assignment of treatment by the study protocol. Samples were immediately processed to separate plasma, which was frozen at −80°C within a few hours of collection.

Metabolite Measurements
Samples were used for quantitative determination of levels of 45 acylcarnitines, 15 amino acids, 5 conventional metabolites (total, low-density lipoprotein, and high-density lipoprotein cholesterol; triglycerides; and glucose), free fatty acids (total, ketones (total and β-hydroxybutyrate), and C-reactive protein as previously described.11 The laboratory was blinded to clinical data from the patients whose samples were being analyzed.

For targeted mass spectrometry-profiled metabolites (acylcarnitines and amino acids), we used previously published methods for sample preparation and analysis.11–13 Proteins were first removed by precipitation with methanol. Aliquots of supernatants were dried and then esterified with hot, acidic methanol (acylcarnitines) or n-butanol (amino acids). Samples were analyzed by tandem mass spectrometry with a TQ Detector (Waters Corp, Milford, MA). The “targeted” intermediary metabolites were quantified by adding mixtures of known quantities of stable-isotope internal standards. Assay ranges are 0.05 to 50 μmol (acylcarnitines) and 5 to 10000 μmol (amino acids). Methodology and coefficients of variation have been previously reported by our group.5,11

Standard analytical chemistry methods were used to determine the concentrations of conventional metabolites (total, low-density lipoprotein, and high-density lipoprotein cholesterol; triglycerides; and glucose) and C-reactive protein using reagents from Roche Diagnostics (Indianapolis, IN) and free fatty acids (total) and ketones (total and β-hydroxybutyrate) using reagents from Wako (Richmond, VA). All assays were performed on a Hitachi 911 clinical chemistry analyzer. Methodology and coefficients of variation have been previously reported by our group.5,11

Statistical Analysis
Our primary goal was to identify individual metabolites and groups of metabolites that changed with administration of heparin. Metabolite levels below the lower limits of quantification were reported as “0,” and any metabolites with >25% of values determined to be “0” were not analyzed. For this reason, there were 7 metabolites (acylcarnitines) that were not analyzed (C6, C12-OH/C10-DC, C18:1-OH/C16-1-DC, C20-OH/C18-DC, C16:1-OH/C14:1-DC, C20:4, and C7-DC). Metabolite levels within patients between the first (preprocedure) sample and second (postprocedure) sample were compared using paired t tests. Differences in preprocedure to postprocedure mean metabolite levels between groups were compared using unpaired t tests. All statistical tests were performed using numeric difference, and both the numeric and percent difference from the first sample were reported. Rank sum tests were used where the samples were not normally distributed as determined by the Shapiro-Wilk test and are denoted by R in the probability values reported in the tables. Demographic and periprocedural variables were compared between the groups using the rank sum test or Fisher exact test of proportion where appropriate. Nominal statistical significance was defined as P≤0.05. No corrections were made for multiple comparisons. All tests were performed using SAS software Version 9.2 (SAS, Cary, NC).

Results

Baseline Clinical Characteristics
In June 2009, 29 patients presenting for cardiac catheterization procedures at Duke University Medical Center were enrolled. Of these, 10 patients (nonheparin group) did not receive heparin during cardiac catheterization and were used as a comparison group to understand changes in metabolite profiles that may occur due to the cardiac catheterization procedure itself. Baseline clinical characteristics in the nonheparin (n=10) and heparin (n=19) groups and indication for cardiac catheterization were similar (Table 1). There were more patients in the heparin group who underwent left heart catheterization. The catheterization procedures performed in each group were otherwise similar. Periprocedural variables, including total time fasting before the procedure, anticoagulation before the procedure, medications administered immediately before the procedure, medications administered during the procedure, the number of patients who required percutaneous coronary intervention, and the type of intravenous contrast that was used, are included in Table 2.

Details of Heparin Administration
For each patient in the heparin group, unfractionated heparin was administered in the catheterization laboratory several minutes after the first (baseline) blood sample was collected as part of usual clinical care (Table 2). Of the 19 patients who received heparin, 16 were given a dose of 1000 U and 3 patients received doses of 3000, 4000, or 5000 U. No patients in the nonheparin group received heparin during the proce-
The median (25th, 75th percentile) dose of heparin administered to patients in the heparin group was 1000 (1000, 1000) units.

### Table 1. Baseline Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>No Heparin (n=10)</th>
<th>Heparin (n=19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.5 (47, 72)</td>
<td>63.0 (55, 72)</td>
<td>0.45</td>
</tr>
<tr>
<td>Sex, no. (percent male)</td>
<td>4 (40)</td>
<td>13 (68)</td>
<td>0.24</td>
</tr>
<tr>
<td>Race, no. (percent white)</td>
<td>7 (70)</td>
<td>14 (74)</td>
<td>0.99</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>31.7 (25, 38)</td>
<td>29.3 (26, 34)</td>
<td>0.66</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.9 (0.7, 1.4)</td>
<td>1.0 (0.9, 1.4)</td>
<td>0.41</td>
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</table>

### Table 1. Continued

<table>
<thead>
<tr>
<th>Procedure performed, no. (%)</th>
<th>No Heparin (n=10)</th>
<th>Heparin (n=19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right heart catheterization</td>
<td>4 (40)</td>
<td>6 (32)</td>
<td>0.70</td>
</tr>
<tr>
<td>Left heart catheterization</td>
<td>3 (30)</td>
<td>18 (95)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Right and left heart catheterization</td>
<td>0 (0)</td>
<td>6 (32)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Continuous variables are shown as median (25th, 75th percentile); categorical variables are shown as percentages. Coronary artery disease was defined by prior myocardial infarction, prior percutaneous coronary intervention, prior coronary artery bypass grafting, or angiographic evidence of coronary artery disease.

The median (25th, 75th percentile) dose of heparin administered to patients in the heparin group was 1000 (1000, 1000) units.

### Table 2. Periprocedural Data

<table>
<thead>
<tr>
<th>Procedure performed, no. (%)</th>
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<th>Heparin (n=19)</th>
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</thead>
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<td>6 (32)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Continuous variables are shown as median (25th, 75th percentiles); categorical variables are shown as percentages. NPO indicates nil per os.
units. The median elapsed times between the first and second blood draws in the nonheparin and heparin groups were 27.0 (22.0, 40.0) and 34.0 (20.0, 39.0) minutes, respectively. The median time elapsed between heparin administration and the second blood draw in the heparin group was 22.0 (17.0, 29.0) minutes. The median activated clotting time value (a measure of the degree of anticoagulation by heparin) obtained at 34.5 (26.0, 45.5) minutes after heparin administration in the heparin group was 146.0 (137.0, 163.0) seconds, consistent with a significant anticoagulation effect.

Metabolomic Data
Levels of 23 individual metabolites changed significantly in the heparin group after heparin administration (Table 3). Levels of 23 metabolites also changed significantly between pre- and postprocedure samples in the nonheparin group. Metabolites that significantly decreased in both the heparin and nonheparin groups include the following: amino acids alanine, aspartic acid/asparagine, histidine, and ornithine; acylcarnitines C10, C10:3, C16, C18, C18:1, and C18:2; C-reactive protein; and low-density lipoprotein and total cholesterol. Additionally, in the nonheparin group there were also significant decreases in the amino acids proline, valine, leucine, isoleucine, methionine, phenylalanine, and tyrosine; acylcarnitines C2, C5:1, and C14:2; and high-density lipoprotein cholesterol. The amino acids glutamine/glutamic acid, valine, leucine, isoleucine, methionine, phenylalanine, and tyrosine; acylcarnitines C3, C8, C10:1, C12, and C14; and triglycerides decreased significantly only in the heparin group. There were no metabolites that significantly increased in the nonheparin group. Metabolites that significantly increased in the heparin group included the amino acid arginine, β-hydroxybutyrate, ketones, and NEFA.

Although there were many metabolite levels that significantly changed from pre- to postprocedure, there were only 4 metabolites in which the mean changes between pre- and postprocedure levels were significantly different between the nonheparin and heparin groups (Table 3). Thus, these metabolites represent those that change in response to heparin and characterization of the kinetics of the cardiac catheterization procedure itself (eg, fasting, sedation, stress, pain, or supine position). These included β-hydroxybutyrate (5.43 versus 66.84 μmol/L; P = 0.009), ketones (21.17 versus 98.49 μmol/L; P = 0.009), NEFA (0.37 versus 1.20 mmol/L; P = 0.017), and triglycerides (−9.33 versus −36.50 mg/dL; P = 0.007).

Discussion
Our goal was to identify individual metabolites and groups of metabolites that changed with administration of unfractionated heparin and that could potentially confound the analyses of metabolic profiles in patients receiving heparin at the time of sample collection. We observed that heparin administration induced measurable changes in peripheral blood metabolite concentrations in samples obtained during cardiac catheterization procedures. Thus, we identified a metabolic signature associated with heparin administration.

This study was necessary because human metabolic profiling studies may be confounded by clinical/environmental variables such as diet and drug effects. Heparin is a possible confounder in cardiovascular metabolic studies. Heparin administration causes the release of endothelial and hepatic LPL, thereby promoting hydrolysis of triglycerides in chylomicrons and very-low-density lipoprotein into 2 NEFAs and monoaoylglycerol. However, the extent to which heparin modulates targeted metabolic profiles obtained during cardiac catheterization procedures had been unknown. These findings are important to investigators working with databases generated from samples obtained during cardiac catheterization procedures, because some patients receive heparin and some do not, and the difference may confound the results of metabolic associations with disease state or clinical outcomes.

Our findings are physiologically plausible and could have potentially been predicted given biochemical principles, among them the fact that heparin administration acutely induces hepatic and endothelial LPL. Compared with patients in the control group, patients who received heparin had a significantly greater decrease in triglyceride levels after heparin administration and a significantly greater increase in NEFAs, which is postulated to be a direct reflection of LPL induction. Furthermore, β-oxidation of NEFAs resulted in increased ketogenesis supported by our observation of significantly greater increases in levels of β-hydroxybutyrate and ketones in patients who received heparin compared with patients who did not. Importantly, there were no other significantly different changes in levels of the metabolites we measured between the heparin and nonheparin groups. This suggests that in patients treated with heparin, associations of these metabolites with outcomes can be interpreted with low risk of confounding by the effects of heparin use.

It is unknown if there is a potential differential response in LPL activity in patients with and without vascular disease. However, the initial heparin effect on LPL was first established >50 years ago in healthy subjects. The same response to heparin and characterization of the kinetics of the LPL response was described more recently in patients receiving heparin for coronary artery bypass surgery. Therefore, our suspicion is low that vascular disease has a major differential effect on metabolic profiles in response to heparin.

Our study was limited by a small sample size. However, similar cardiovascular metabolic analyses have achieved adequate power using samples sizes in this range. The analysis was also confined to a limited number of metabolites reflective of the activity of most key metabolic pathways. The human metabolome is composed of thousands of metabolites, and it is possible that other metabolites that were not investigated may have significantly changed between the heparin and nonheparin groups. Variability in sample collection or processing could have affected the results. However, we used a standardized protocol to limit this type of variability, and all samples were processed at the same time. Finally, although variation in the precision of the assays we used could have influenced the metabolites that we detected as changing differentially between the heparin and nonheparin groups, we feel this is unlikely because the coefficients of variation across our mass spectrometry and conventional assays are similar.
### Table 3. Metabolites for Which Pre-/Post-procedure Changes Were Different Within Either the No Heparin or Heparin Groups

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>No Heparin</th>
<th>Heparin</th>
<th>Mean</th>
<th>SD Preprocedure</th>
<th>SD Postprocedure</th>
<th>Mean</th>
<th>SD Preprocedure</th>
<th>SD Postprocedure</th>
<th>Difference</th>
<th>Between Groups</th>
<th>Mean</th>
<th>SD</th>
<th>%</th>
<th>P</th>
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<tr>
<td>ALA</td>
<td>10</td>
<td>9</td>
<td>163.3 ± 22.2</td>
<td>132.4 ± 20.8</td>
<td>185.4 ± 21.2</td>
<td>0.21 ± 0.02</td>
<td>103.9 ± 21.3</td>
<td>129.2 ± 20.2</td>
<td>0.34</td>
<td>0.53</td>
<td>0.67</td>
<td>0.56</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>ASX</td>
<td>10</td>
<td>9</td>
<td>232.1 ± 28.6</td>
<td>216.3 ± 27.2</td>
<td>246.7 ± 28.1</td>
<td>0.21 ± 0.02</td>
<td>227.8 ± 26.9</td>
<td>250.1 ± 27.5</td>
<td>0.34</td>
<td>0.53</td>
<td>0.67</td>
<td>0.56</td>
<td>0.79</td>
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<tr>
<td>PRO</td>
<td>10</td>
<td>9</td>
<td>54.3 ± 6.3</td>
<td>52.1 ± 5.9</td>
<td>56.8 ± 6.0</td>
<td>0.21 ± 0.02</td>
<td>53.2 ± 5.8</td>
<td>58.3 ± 6.1</td>
<td>0.34</td>
<td>0.53</td>
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<tr>
<td>VAL</td>
<td>10</td>
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<td>19.3 ± 2.1</td>
<td>18.3 ± 1.9</td>
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<td>20.2 ± 2.0</td>
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<tr>
<td>LEUCINE</td>
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<td>9</td>
<td>136.2 ± 15.4</td>
<td>130.5 ± 14.9</td>
<td>141.8 ± 15.6</td>
<td>0.21 ± 0.02</td>
<td>132.9 ± 14.3</td>
<td>145.3 ± 15.0</td>
<td>0.34</td>
<td>0.53</td>
<td>0.67</td>
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<td>PHE</td>
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<td>80.2 ± 7.2</td>
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<tr>
<td>ARG</td>
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<td>186.6 ± 21.4</td>
<td>181.3 ± 19.8</td>
<td>192.5 ± 22.0</td>
<td>0.21 ± 0.02</td>
<td>181.8 ± 19.6</td>
<td>195.0 ± 21.3</td>
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<tr>
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<td>12.0 ± 1.3</td>
<td>0.21 ± 0.02</td>
<td>10.8 ± 1.0</td>
<td>12.4 ± 1.2</td>
<td>0.34</td>
<td>0.53</td>
<td>0.67</td>
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<tr>
<td>PRO</td>
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<td>216.4 ± 23.1</td>
<td>209.3 ± 21.7</td>
<td>223.5 ± 23.8</td>
<td>0.21 ± 0.02</td>
<td>210.5 ± 22.2</td>
<td>226.6 ± 24.0</td>
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</tbody>
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Notes:
- ALA: aspartic acid.
- ASX: aspartic acid.
- PRO: proline.
- VAL: valine.
- LEUCINE: leucine.
- PHE: phenylalanine.
- TRP: tryptophan.
- HIS: histidine.
- ARG: arginine.
- GLY: glycine.

**ALMA indicates alanine; ASX, aspartic acid; PRO, proline; VAL, valine; LEU, leucine; LEUCE, leucine; MET, methionine; PHR, phenylalanine; TRP, tryptophan; HIS, histidine; GLU, glutamic acid and glutamine; ARG, arginine; ORN, ornithine; HDL, high-density lipoprotein; and LDL, low-density lipoprotein. (R) denotes metabolites that were nonnormally distributed, and thus, a rank-sum test was used.**
Our results could have been confounded by other factors in addition to heparin; however, the use of a nonheparin comparison group allowed us to “subtract” the effect of the cardiac catheterization itself on metabolite profiles (eg, changes induced due to stress of the procedure, fasting, sedation, supine position, or pain). Patient demographics, comorbidities, home and periprocedural medications, time fasting, and indication for procedure were similar in the heparin and nonheparin groups, and our use of a pre-/postdesign for each group should also minimize the concerns of additional confounding differences between study groups. There were small differences between the heparin and nonheparin groups, but we believe that these differences were unlikely to have a substantial effect on our findings.

In summary, we have identified differences in metabolite levels between patients who received heparin during cardiac catheterization and those who did not. Our findings are physiologically plausible despite the inherent limitations in our study. These findings are important to allow appropriate interpretation of metabolomic studies in populations exposed to heparin.

Acknowledgments
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Disclosures
None.

References

CLINICAL PERSPECTIVE
In the hopes of identifying novel biomarkers and mechanisms of cardiovascular disease development, studies are increasingly using novel molecular profiling techniques such as metabolomics and proteomics. For example, we have successfully used metabolomic profiling to identify metabolic signatures that discriminate individuals with coronary artery disease. However, such studies in human blood samples can be confounded by environmental and other external factors. In particular, medications such as those commonly used in cardiovascular disease treatment can confound the association between biomarkers and disease. In fact, heparin has a known lipolytic effect and is commonly used for cardiovascular disease treatment. In this study, we examined the effect of systemic heparin administration on 63 metabolites and standard lipids using a targeted, quantitative mass spectrometry-based metabolomic platform in a cohort of patients referred for cardiac catheterization. As hypothesized, we found that the only metabolites that significantly changed with heparin administration were those related to the known lipolytic effect of this medication (ketones, β-hydroxybutyrate, nonesterified fatty acids, and triglycerides). These findings are important for accurate interpretation of future metabolic profiling studies in populations who may be exposed to heparin.
Effect of Heparin Administration on Metabolomic Profiles in Samples Obtained During Cardiac Catheterization

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